Integrating -Omics: Systems Biology as Explored Through *C. elegans* Research

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http://dx.doi.org/10.1016/j.jmb.2015.03.015
Edited by S. Koide

Abstract

-Omics data have become indispensable to systems biology, which aims to describe the full complexity of functional cells, tissues, organs and organisms. Generating vast amounts of data via such methods, researchers have invested in ways of handling and interpreting these. From the large volumes of -omics data that have been gathered over the years, it is clear that the information derived from one -ome is usually far from complete. Now, individual techniques and methods for integration are maturing to the point that researchers can focus on network-based integration rather than simply interpreting single -ome studies. This review evaluates the application of integrated -omics approaches with a focus on *Caenorhabditis elegans* studies, intending to direct researchers in this field to useful databases and inspiring examples.

Introduction

Since the first whole genome (of the bacteria *Haemophilus influenzae*) was sequenced by Fleischmann *et al.* in 1995 [1], researchers increasingly recognized the informational wealth of large-scale, high-throughput data. This meant the start of the development of multiple -omics fields, which focus on the presence or behavior of large groups of biomolecules of a kind within a sample. Nowadays, more than 30 -omics fields are described. Among these, genomics, transcriptomics, proteomics and metabolomics are the most mature and most studied ones. In addition, an increasing number of lesser known and more refined -omics fields, like epigenomics, secretomics, glycomics and lipidomics, have also come to the attention of researchers in biomedical and biological fields [2].

Data from different -omics studies are often categorized into three main types: components data, interactions data and data from functional states analyses (Fig. 1) [3]. Components data cover descriptive studies of a defined part of a biological sample. Genome annotation, transcriptome, proteome, metabolome, glycome and lipidome data—be it descriptive or differential—are just a few examples. This is in contrast to interactions data, in which the relationships between components within a sample are investigated. Two types are particularly popular here: protein–protein and protein–DNA interactions, in general referred to as interactomics studies. Such data provide important scaffolds for the elucidation of biological networks. The last category is more vaguely defined and describes the overall behavior or phenotype of a biological system. Fluxomics and phenomics studies belong to this class of -omics data [3].

-Omics approaches aim to address the multifactorial origins of biological systems or diseases by gathering data on as many targets as possible rather than "simply" looking for a few critical players within a single biological component. However, even at this scale, the information often comes in scattered bits and pieces [4–7]. This has led us to an increased interest in the integration of multiple complementary components, interactions or functional states datasets—a practice sometimes referred to as "integromics". It should be noted that, based on such elaborate

Please cite this article as: Van Assche Roel, et al, Integrating -Omics: Systems Biology as Explored Through *C. elegans* Research, J Mol Biol (2015), http://dx.doi.org/10.1016/j.jmb.2015.03.015

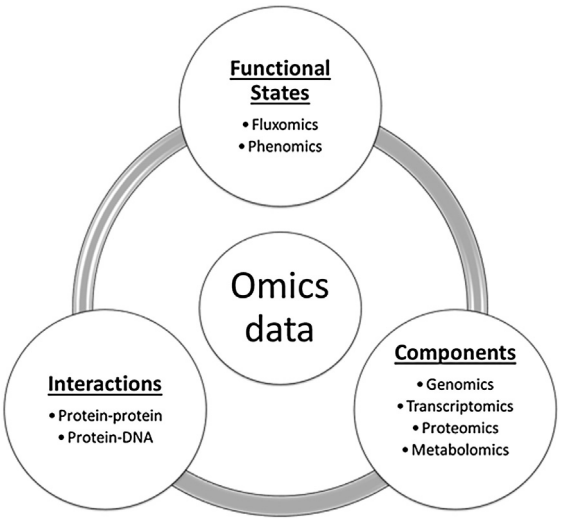

Fig. 1. General types of -omics data and examples (adapted from Ref. [3]).

information, the critical players often emerge as key nodes of complex biological networks [8–10]. Integration of the interactions between biological entities has evolved to the field of systems biology, wherein a holistic approach is used to understand complex biological systems. In this review, we summarize the recent advances in the field, focusing mainly on *Caenorhabditis elegans* research.

C. elegans is a small free-living roundworm, found primarily in environments rich in bacteria such as compost and rotting fruit [11]. Sydney Brenner introduced it in the 1960s for the study of its development and nervous system [12]. Praised by many as an ideal model organism due to the ease by which it can be reared in laboratory environments, *C. elegans* has provided a multitude of key insights into molecular biology (molecular mechanisms underlying RNAi) [13], central principles in cell biology (apoptosis as being essential to development [14]) and neurobiology (genetics of axonal guidance [15]). *C. elegans* was the first multicellular organism to have its genome sequenced [16], and extensive bioinformatics and genetic tools exist to facilitate research using this system. In addition to its relatively low complexity, many well-kept *C. elegans* -omics databases (Table 1) support its use in the study of complex molecular networks in systems biology. Especially in light of -omics studies, this nematode has multiple practical advantages for standardization of experiments (precise age synchronization, identical amount of cells, no unwanted influence of sex in hermaphrodite cultures and strict control over nutritional content). In this way, the introduction of undesirable experimental variation is kept to a bare minimum when compared to experiments with most other complex organisms.

Why Integrate?

Biological systems are neither static in time nor homogenous in spatial distribution [2]. Every -ome analysis represents a snapshot of the physiological state at the time the -ome is extracted. Beside differences inherent to the biology and biochemistry of each sample, the development of -omics methods strongly depends on the technical evolution of the instruments needed, each with their respective limitations. For example concerning transcriptomics studies, nucleic acid modifications and splice variants may not be observed, transcripts may fail to bind (or cross-hybridize with) microarray probes and RNAseq techniques encounter problems concerning coverage in repeat-rich regions. More downstream, enrichment methods for specific proteins are being developed [17], but no amplification method exists for these extracted molecules. Some efforts have already been made in *C. elegans* with co-immunoprecipitation of protein complexes to detect low abundant features [17]. However, in complex samples, low abundant features are hard to detect in mass spectrometry-based studies, not even mentioning that extraction methods only result in partial representation of the whole -ome. On top of that, the use of database-driven identification is limited to known database entries, often leading to lists of “interesting unknowns”. This issue can be overcome, to some extent, using *de novo* methods, but these are time consuming and especially for metabolomics studies often put on the back burner.

Because each -omics field is characterized by its own strengths and weaknesses and is therefore bound to miss out on a part of the complexity of a biological system, a systems biology approach is usually based on the data integration of two or more -omes. The goal of integration is twofold: on the one hand, a more accurate picture of the behavior of one species of molecules is obtained, and on the other hand, a more holistic view of the system is achieved by looking at the behavior of multiple components (RNAs, proteins, etc.). This assists in the formulation of system-wide biological hypotheses. For example, Walhout *et al.* combined interactome, phenome and transcriptome data for the *C. elegans* germline and found that essential proteins have a tendency to interact with each other (phenome + interactome) and that pairs of genes encoding interacting proteins tend to exhibit similar expression profiles (interactome + transcriptome) [73].

In the quest for a complete description and annotation of a single -ome, different technical platforms are often combined. Such an approach has not yet been applied to *C. elegans* research, but valuable insights were delivered in a study focusing on NCI-60 cancer cell line ovarian tumors [18]. Here, approximately half of all genes detected in the samples using four different transcriptomics

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Table 1. A collection of online available *C. elegans* -omics databases (adapted from [31]).

Name database	URL	Information in database	Data category
WormBase	www.wormbase.org/	Biology of *C. elegans*; central database including a wide array of information (e.g., expression, RNAi, ORFs, phenotypes, interactions)	Components data Interactions data Functional states data
WORFDB	http://worfdb.dfci.harvard.edu/	ORFeome of *C. elegans*	Components data
Hope Laboratory Expression Pattern Database	http://worfdb.dfci.harvard.edu/promoteromedb/	PROMOTEROME of *C. elegans* (promoters fused to GFP expression patterns)	Components data
WormAtlas	http://www.wormatlas.org/	Anatomy and behavior of *C. elegans*	Components data Interactions data Functional states data
WormImage	http://www.wormimage.org/	Electron microscopy images of *C. elegans*	Components data
C. elegans Gene Knockout Consortium	http://celeganskoconsortium.omrf.org/	Gene knockout strains	Functional states data
RNAiDB	http://www.rnai.org/	Phenotypes of many RNAi experiments	Functional states data
PhenoBank	http://www.worm.mpi-cbg.de/phenobank/cgi-bin/MenuPage.py	RNAi phenotypes	Functional states data

platforms were found in all datasets [18]. Such combinatory technical approaches are primarily popular in metabolomics research, where they ensure a broad coverage of the quite complex set of metabolites present in each sample [19,20].

With the aforementioned limitations in mind, it is easy to understand that a combination of different techniques involves multiple advantages [5]. If the data share a common correlation over the different -omics analyses, the obtained results will be more robust compared to single -ome analysis. However, also less correlated identifiers can deliver new insights, for example, pointing toward post-transcriptional or post-translational regulation [21–23]. For example, if the amounts of proteins and transcripts are not correlated, some post-transcriptional regulation might be influencing the protein level data. Such discrepancies can lead to new insights in the regulation of certain genes. Additionally, certain identifiers are often missed at a single-ome dataset. These might yet be retrieved from a different biological entity to complement the set of biologically relevant information [11,15]. Combining multiple -omics data is therefore assumed to improve on pathway enrichment analyses [24].

-Omics Data

Components data

Components data are gathered with the intention of describing an organism's set of biomolecules as fully as possible. This may be under standard conditions [25], for specific conditions of interest [26] or in comparative studies [20,27,28]. Among these, differential components usually look at network changes under specific perturbations, such as the effect of drug compounds on the proteome of *C. elegans* [29] or the effect of a toxic peptide on the metabolome of *C. elegans* [20]. The ease of working with *C. elegans* in the field of the systems biology is that, nowadays, many online databases collect subsets of the abundant *C. elegans* components data (Table 1), for example, WORFDB, a resource of all protein-encoding ORFs[‡] (*o*pen *r*eading *f*rames). Based on ORF predictions in databases such as WormBase[§], up to 22,000 ORFs were verified [30] using sequencing-based methods (using expressed sequence tags and gateway clones) [31], which are now available through WORFDB. In addition, innumerate transcriptomics experiments have been performed in *C. elegans* [32–34]. Microarrays and serial analysis of gene expression are still popular, but the field is shifting to the use of the more sensitive RNA sequencing methods. These have been applied to whole transcriptome profiling, ribosomal profiling and RNA editing studies in *C. elegans* [35]. Once more, the use of a model system is emphasized for this sort of research as an advantage because of the availability of up-to-date gene annotations. An extension of the ENCODE project, ModENCODE, is a comprehensive encyclopedia of genomic “functional elements” of the model organisms *C. elegans* and *Drosophila melanogaster.* It provides access to abundant data on domains of gene structure, mRNA and non-coding RNA expression profiling; transcription factor binding sites; histone modifications and replacement; chromatin structure; DNA replication initiation and timing and so on [36]. These abovementioned databases are just a selection of the existing *C. elegans* databases (Table 1).

More downstream, both proteomics [28,29] and peptidomics (see *C. elegans* review [37]) are routinely used in *C. elegans* research. Nowadays, these are extended to more specialized analyses such as phosphoproteomics [38]. Data of these latter -omics

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fields can be found on platforms such as WormBase at this moment, but a user-friendly database in which all data are centralized is missing. At last, metabolomics, the youngest and most downstream -omics field, is starting to be used more frequently [20] in *C. elegans* research. Unfortunately, the *C. elegans* community lacks a proper platform with specific endogenous metabolites or related information.

Interactions data

Many biomolecules do not function as isolated entities but rather within complexes. Interactions data comprise information about interactions between molecular components (generally protein–protein or DNA–protein interactions), often referred to as the interactome.

The interactomics field mainly revolves around protein data. This is because proteins are considered the central regulators of the biochemical reactions supporting organismal life and have a variety of functions (such as enzymatic, structural, storage, transport, transcription factor or immune system functions), which they tend to execute in complexes, either with DNA (e.g., transcription factors) or with other proteins. It should be noted that there is now a growing interest in functional non-coding RNAs as well; while their numbers are still debated, non-coding RNAs may direct transcription and translation (as reviewed by Shapiro [39] and Sabin *et al*. [40]).

Protein–protein interactions are often studied by yeast two-hybrid techniques in which researchers screen for interacting proteins in a high-throughput manner. Such an approach has helped uncover, for example, regulatory networks in the *C. elegans* DAF-7/TGF-β signaling system [41]. Such *C. elegans* interactions data, including two high-throughput and large-scale yeast two-hybrid screens [42,43], can easily be consulted in the worm interactome database[ll] [43].

Interactome data in *C. elegans* are not merely limited to protein–protein interactions. Many high-throughput DNA–protein interaction studies have been performed as well—typically using chromatin immunoprecipitation. Optimized methods for the global identification of transcription factor binding sites in *C. elegans* (through ChIPseq) are well-described in literature [44]. Chromatin-immunoprecipitation-based techniques have been used for the elucidation of other DNA–protein interactions as well, including interactions between modified histones and DNA to study epigenetic control of transcription and epigenetic inheritance in *C. elegans* [45–48].

One recent advancement in *C. elegans* interactomics is a method to directly identify non-coding micro-RNA target sites, paving the way for a better understanding of how the micro-RNAs act to alter cellular biology [49].

Functional states data

Functional states data comprise phenomics and fluxomics data. *C. elegans* has a well-characterized phenome that is built up from data often retrieved through high-throughput RNAi screens. These have been performed for nearly every protein-coding gene and a variety of phenotypes, of which the data are available in different databases (RNAiDB, PhenoBank and WormBase) [50]. The power of such screens and richness of data is demonstrated, for example, in a genome-wide RNAi screen for modifiers of polyglutamine aggregation in *C. elegans* [51]. Teuling *et al*. discovered 186 genes that suppress the polyglutamine aggregation process [51]. Starting from these data, a list of 26 homologous human genes was selected in order to further unravel the function concerning protein aggregation.

RNAi screens not only do predict the effect of an individual gene but could also provide information about interactions of functionally related proteins. For example, if some RNAi knockdowns result in a common phenotype, called a phenocluster, interactions can be predicted or novel protein functions can be discovered [31]. In this way, a somewhat focused list of candidate interaction partners can be proposed, which then need to be verified in great detail [52,53]. Many such screens are dependent on an automated analysis of the phenotype, which is essential to add robustness and sufficient throughput to these RNAi screens [54]. Some systems specific to *C. elegans* were developed to facilitate the automated detection of phenotype, often using locomotion as readout [55].

In addition to phenomes, *C. elegans* has also been used to generate fluxomics data. These studies often rely on the use of stable isotopes as markers to follow the dynamics and turnover of metabolic processes over time. Metabolic flux studies have been performed to evaluate metabolic responses to cadmium administration [56] or mitochondrial dysfunction [57] in *C. elegans*.

Synergistic Effects of Combinatorial Analyses

Although one single -omics dataset can yield clear biological insights, combination of multiple -omics data can lead to synergistic findings. To date, the most popular differential combinatorial analyses have relied on proteomics and transcriptomics experimental input. It is known that the correlation between mRNA and protein levels is insufficient to predict protein expression levels. Transcript and protein expression levels for selected genes expressed in the yeast *Saccharomyces cerevisiae* were determined [58]. These results showed a lack of a 1:1 correlation between mRNA and proteins and

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prompted researchers to combine both -omes in one analysis [5,58–65]. Experiments can be biased by methodological constrains and technical limitations, leading to a decreased mRNA–protein correlation [66]. Such low correspondence can be caused by several factors, such as post-transcriptional regulation that can account for up to 50% of the discordance between mRNA and protein quantities [61,67]. One example of such discrepancies can be found in a study evaluating the molecular effects of lifespan-extending interventions in *C. elegans* [21]. A direct correlation between mRNA and protein levels was observed for genes concerning *S*-adenosyl methionine synthesis, muscle-related proteins and branched-chain amino acid degradation. However, an increase in abundance of ribosomal subunits was observed in the proteomic analysis that was absent in the transcriptome. Although the differences between mRNA and protein levels can be due to technical limitations, in some cases, real biological insights can be obtained from comparative analysis of different -omics data (such as specific post-transcriptional regulation of ribosomal subunits in long-lived worms [21]).

Also, information from one -ome can be used to complete information of the other. An example of this is the field of proteogenomics, an area of research at the interface of proteomics and genomics. Here, proteomics data are used to identify and characterize novel protein-coding genes. Applications can be found in reading-frame determination, identification of gene and exon boundaries, evidence for post-translational processing, identification of splice forms including alternative splicing and, also, prediction of completely novel genes [68]. Databases to facilitate proteogenomic analyses have been constructed using *C. elegans* RNAseq data [69]. These analyses in *C. elegans* improve the annotation of unknown genomic regions and provide information about splicing events in transcribed regions. Such methods can be especially helpful in the specific case of other species with poorly or unannotated genomes, where peptidomics or proteomics data can be matched to the unannotated genome to assist in further gene identification [70].

When multiple -omes are analyzed within one study, decisions need to be made on the way the emerging data will be treated. Integration can be performed in a rather naïve way by simply comparing the different outcomes from each -ome, but this can more aptly be executed with dedicated bioinformatics methods. While still less used due to their advanced nature, researchers are increasingly recognizing the value of such advanced statistical and bioinformatics approaches.

Also for *C. elegans* studies, the majority still relies on the naïve method mentioned above to combine multiple -omics datasets. Here, each -ome is analyzed separately (Fig. 2a) and the final combination is performed in a *post hoc* manner [71]. For example, protein products of genes with similar expression patterns can be candidate interaction partners, as shown by research looking for DNA damage responses [72] and the fundamental germline biology [73]. Basic integration of *C. elegans* -omics data is also of value to drug screening: the genome and phenome of the worm have been used to screen for candidate drug targets in nematodes. This reduction in candidate target genes is expected to facilitate further screenings [74].

Pending a further evolution toward more complex integrative methods, researchers complement and compare individual experimental outcomes in meta-analyses to obtain a more complete view on their question of interest. This is demonstrated for -omics data regarding innate immunity in *C. elegans* [75] in which multiple studies subscribe the use of this model system to study protective responses against invading pathogens. Thanks to integration of these studies, some protein families (lectins, lysozymes, collagens and peptidases are just few examples) were discovered to play an important role in the immunity of *C. elegans*. These studies joined different data with the aim to find new insights, which is to a certain extent possible without relying on further bioinformatics interventions.

Statistics and Bioinformatics Tools in -Omics Data Analysis and Integration

Although direct comparative methods (Fig. 2a) are straightforward, they fail to integrate correlation or pathway information over the different -omes within a functional biological context. Therefore, analyses are shifting to more sophisticated approaches. For example, different molecular entities can be modeled using multivariate probability models (Fig. 2b). Since the measured effects in a single -ome dataset can be adjusted by data from other components, data incorporation from multiple molecular components can strengthen the statistical power. An even more elaborate integrating method relies on network-based integration (Fig. 2c), in which the simple one-to-one comparison of datasets is replaced by mapping all data onto molecular networks [71]. One such study relied on transcriptomics, protein–protein interaction and high-content phenotypic data [42,76,77] to formulate a hypothesis of the molecular functioning of embryonic development in *C. elegans* [78]. Thanks to a dedicated computational method, a more reliable list of genes predicted to function in this network could be proposed for further *in vivo* verification.

Integration of -omics derived data is driven by mathematical models and computational tools to maximize the generation of complementary information [79]. Here, *C. elegans* researchers can rely on

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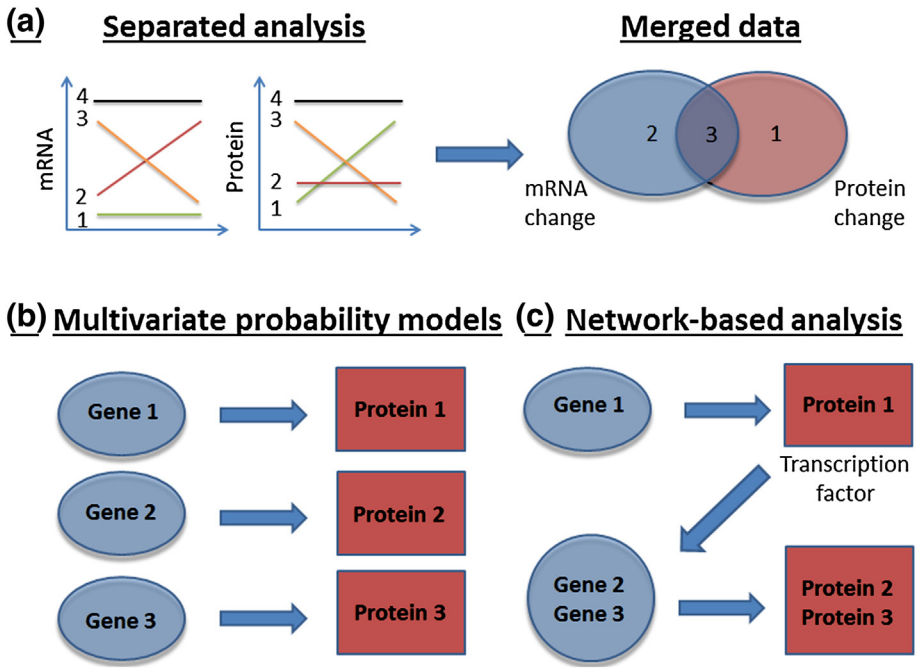

Fig. 2. The three most used methods for the integration of multiple -omics data [71].

efforts made by others within different organismal contexts. Any approach moving away from a simple data merging (Fig. 2a) showed to significantly increase on overlap between -omes and interesting target identification [4,24,80]. In this way, biological interpretation options increase significantly [71].

In order to overcome the mainly bioinformatics obstacles in such analyses, there are some software packages already available (Pointillist [81], 3Omics [82], Paintomics [83] and KaPPA-View [84]). These packages (Table 2) can handle missing values and reconstruct the cellular network—it is therefore to be hoped that their use will increase in the *C. elegans* community, which is rich in data to support such analyses.

Challenges and Future Perspectives

Although integromics can be valuable to provide a more holistic overview of regulatory pathways within a biological system, many challenges still have to be overcome in order to produce unambiguous results. Main challenges are the inherent complexity of the biological systems, the lack of sufficient, proper bioinformatics tools and the large amounts of data [85].

One of the main characteristics of -omics research is the generation of huge asymmetric datasets. Although integration yields a lot more information, dealing with such “big data” remains an important challenge in statistical analysis. In addition, -omics data are prone to a high degree of variation, supported by the observation that different technical platforms within one -ome analysis usually result in widely varying data [24]. It is evident that this intra-ome variation impedes to some extent on the inter-ome integration. The primary data source can also impose technical challenges on integrative efforts: due to the different nature of diverse -omics platforms, differences in type of primary data format impede on a straightforward integration [22,24]. This can generally be overcome by converting raw data formats into a suitable format to ensure their successful import into integration software.

Furthermore, proper integromics analyses have to deal with the lack of common annotated names for corresponding compounds across the different -omes. For instance, it can be difficult to combine gene and protein names within one analysis, as these databases need to be linked to support functional network analysis. By converting the identifiers in representative gene names, Kohl *et al.* (2013) could link 50% of the proteins to transcripts in patients suffering from hepatocellular carcinoma [86]. This is in contrast to less than 15% linking without conversion of the identifiers in representative gene names.

Depending on the hypothesis, it may suffice to use a targeted, single -ome approach. A part of the -omics field is moving toward hypothesis-driven -omics studies (asking how an intervention influences a specific biological pathway), which are not always in need of integrated approaches. In *C. elegans* research, targeted proteomics has been executed to validate predicted micro-RNA targets [87]. However, for those

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Table 2. An overview of software packages used for multiple -omics integration.

Levels	Methods	Software	Freeware	Reference
Any combination of -omics results	/	Pointillist: weighted integration of *P* values, no network info	Yes	Hwang *et al.* (2005a) [65]
Genomics, transcriptomics, proteomics (mRNA and protein abundance, genome-wide protein–DNA interaction, protein–DNA and protein–protein interactions)	Integration of known databases that pertain localization of protein–protein and protein–DNA interaction	Pointillist: weighted integration of *P* values, no network info	Yes	Hwang *et al.* (2005b) [69]
Transcriptomics, proteomics, phosphoproteomics	Microarray, label-free LC-MS/MS	Weighted integration of fold changes, adjusted for dataset size	Yes	Balbin *et al.* (2013) [67]
Transcriptomics, proteomics	Transcript and proteomic expression profiles	Integration of co-expression network from different -omics levels	/	Gibbs *et al.* (2014) [74]
Transcriptomics, proteomics, metabolomics (only human data)	/	3Omics one-click Web tool: correlation networking, co-expression, phenotyping, pathway enrichment, and Gene Ontology enrichment	Yes	Kuo *et al.* (2013) [70]
Transcriptomics, metabolomics	/	Paintomics: pathway enrichment of metabolomics and transcriptomics	Yes	García-Alcalde *et al.* (2011) [71]
Transcriptomics, metabolomics (plant specific)	/	KaPPA-View	Yes	Tokimatsu *et al.* (2005) [72]
Transcriptomics, proteomics, metabolomics	/	VANTED	Yes	Junker *et al.* (2006) [75]
Transcriptomics, proteomics, metabolomics	/	ProMeTra: mapping on defined pathways	Yes	Neuweger *et al.* (2009) [76]
Genomics, transcriptomics, proteomics, metabolomics	/	MAYDAY (including ChromeTracks tool)	Yes	Symons *et al.* (2010) [77]
Genomics, transcriptomics, proteomics, metabolomics	/	PaVEsy	Yes	Lüdemann *et al.* (2004) [78]
Transcriptomics, proteomics, interactomics	/	SteinerNet: interactions between transcriptomic and proteomic analysis investigated with prize-winning Steiner tree algorithm	Yes	Tuncbag *et al.* (2012) [79]
Genomics, proteomics	Nano-HPLC + LTQ velos orbitrap pro-MS, RNAseq	Samifier tool (tool to enable a nexus between proteomic and genomic analysis), PG Nexus pipeline	Yes	Pang *et al.* (2013) [80]
Transcriptomics, proteomics, metabolomics	/	IntegrOmics (an R package to unravel relationships between two -omics datasets using canonical correlation analysis and partial least-squares regression)	Yes	Lê Cao *et al.* (2009) [81]

Abbreviations used: LC-MS/MS, liquid chromatography tandem mass spectrometry; nano-HPLC, nano-high-performance liquid chromatography; LTQ, linear trap quadrupole.

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using -omics approaches in an exploratory, hypothesis-formulating way, it is to be expected that integrated -omics studies will become the golden standard, enabled by the rapidly growing technical evolutions in each of the -omics fields.

-Omics analyses will also become less expensive, an important factor in making combined analyses more accessible for many research teams. With the more recent development of single-cell analysis techniques [88], cellular heterogeneity—a common issue in systems biology—can be minimized. This exemplifies how (integrated) -omics techniques keep evolving at the forefront of science, and this continues to support innovative research.

Conclusion

Multiple components of complex biological systems can be studied using high-throughput -omics techniques. In addition, integration of the corresponding -omics data provides a more comprehensive overview of the full complexity of biological systems, formerly more difficult to unravel. The progress in bioinformatics solutions supports these systems biology approaches and demonstrates the potential of integrating multiple -omics data. *C. elegans* research benefits from several high-quality and properly maintained -omics databases. As exemplified by a limited but valuable number of integrating -omics studies using this model system, we would like to encourage others in the field to consider such approaches for which they can rely on several bioinformatics tools enlisted in this review. While some challenges remain, implementation of integromics approaches in many fields in the future is a must.

Acknowledgments

Funding for this project was provided by Interuniversity Attraction Poles (IAP-P7/44). V.B. is an IWT Flanders research fellow. L.T. is an FWO--Flanders postdoctoral fellow. W.D.H. is funded by the KU Leuven Research Fund.

Received 24 December 2014;
Received in revised form 24 March 2015;
Accepted 25 March 2015
Available online xxxx

Keywords:
systems biology;
integrating -omics;
C. elegans

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‡ http://worfdb.dfci.harvard.edu/
§ www.wormbase.org
‖ http://interactome.dfci.harvard.edu/C_elegans/

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